correlating the increase in fluorescence intensity or in colorimetric intensity ~~optical signal~~ generated upon the signaling aptamer binding the ligand with the quantity of ligand bound to the signaling aptamer.

REMARKS

Status of the application

Applicants filed a Notice of Appeal on May 27, 2003 as a response to the Response after Final filed April 22, 2003 had not been received. Upon receipt an Advisory Action mailed July 14, 2003 which sets the period for reply to expire nine months, i.e., August 26, 2003, from the mailing date of the final rejection, Applicants wish to file a Request for Continuation and the instant supplemental response in lieu of an Appeal Brief.

Status of the claims

Claims 1-2, 5-12, 15, 17-25 and 28 are pending. Claims 1-2, 5-12, 15, 17-25 and 28 are rejected. Claims 1, 6-7, 10, 15, 20, and 28 are amended herein. Claims 2-5, 13-14, 16-18, 26-27 are canceled. No new matter has been added.

Amendments to the claims

Independent claims 1 and 15 are amended to overcome prior art rejections under 35 U.S.C. 102(b) & 102(e) and 103(a) as discussed *infra*. Additionally, Applicant has amended the preamble of claims 1 and 15 to recite transducing the conformational change of the ligand upon binding the signaling aptamer to a detectable increase in a fluorescence intensity or colorimetric intensity signals (claim 1) and detectable increase in fluorescence intensity or colorimetric intensity (claim 15) and deleted this limitation from the body of the claims. Claim 2 was canceled to overcome the 35 U.S.C. 112, second paragraph rejection. Claims 6-7, 10 and 20 were amended to correct dependency. No new matter has been added.

The 35 U.S.C. §112, second paragraph rejections

Claim 2 is rejected under 35 U.S.C. §112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention in that the recitation "further comprises an electrochemical signal or an enzyme signal" lacks antecedent basis. Applicant respectfully traverses this rejection. Applicant has canceled claim 2 thereby rendering the rejection moot.

Accordingly, Applicant respectfully requests that the rejection of claim 2 under 35 U.S.C. § 112, second paragraph, be withdrawn.

The 35 U.S.C. §102(b) & (e) rejections

Claims 1-2, 5-12, 15, 17, 19, 23, and 25 are rejected under 35 U.S.C. §102(b) as being anticipated by **Pitner** et al. (U.S. 5,650,275). Claims 1-2 and 7-12 are rejected under 35 U.S.C. §102(b) as being anticipated by **Gold** et al. (U.S. 6,242,246). Applicant respectfully traverses these rejections.

Regarding **Pitner** *et al.* and **Gold** *et al.* as applied to claim 1 and **Pitner** *et al.* as applied to claim 15, the Examiner states that these references disclose the claimed invention method of detecting a differential/fluorescence signal of a signaling aptamer (detectably labeled nucleic acid ligand/fluorescent labeled polynucleotide) upon binding a ligand (target molecule), the differential/fluorescence signal generated by a reporter molecule/fluorescent dye (spectroscopically detectably labeled nucleic acid ligand/fluorescent label) comprising the steps of contacting (mixing) the signaling aptamer (spectroscopically detectably labeled nucleic acid ligand/fluorescent label) with the ligand (target compound) wherein the former binds (complexes

with) the latter and detecting the differential signal/fluorescence signal generated by the reporter molecule/fluorescent dye (spectroscopically detectably labeled nucleic acid ligand/fluorescent label measured before and after binding) where the differential/fluorescence signal is expressed as fluorescence intensity (Pitner: col. 5, ll. 22-24; col. 13-14; claim 1 and **Gold** *et al.*: Abstract, ll. 2-14, col. 15, ll. 49-53, col. 16, ll. 54-57).

Additionally, the recitation in the instant claims of "transducing the conformational change of a signaling aptamer that occurs upon binding the signaling aptamer binding a ligand to a detectable optical signal generated by a reporter molecule/fluorescent dye that is appended to the signaling aptamer at a site that does not interefere with a ligand-binding site of the signaling aptamer prior to binding the ligand" is inherent in the claim 1 method of **Pitner** *et al.* and of **Gold** *et al.* It was known in the prior art (see Description of the Related Art in the instant application) that aptamers undergo an induced fit conformational change in the presence of their cognate ligands and thus an appended dye easily undergoes a ligand-dependent change in its local environment (**Pitner** *et al.*: col. 2, lines 55-59; col. 11-12, Ex. 5 and **Gold** *et al.*: col. 15, lines 49-52, col. 16, lines 54-56).

Pitner *et al.* teach a method of detecting a target compound in a sample by measuring the fluorescent polarization or fluorescent anisotropy of a fluorescently labeled receptor molecule and subsequently measuring these values when the receptor molecule is placed in solution with the target compound. The labels are attached to the receptor molecule by chemical coupling of suitable reactive derivatives of the label molecule to suitable linkers or tethers. Commercial examples of these are amino hexyl or amino propyl linkers (col. 4, ll. 22-26).

Gold *et al.* disclose a nucleic acid ligand biochip having a solid support to which one or more specific nucleic acid ligands is attached in a spatially defined manner. The nucleic acid ligands are contacted by a target molecules and if the target is bound by the nucleic acid ligand or receptor a detectable change occurs which is a change in fluorescence or a change in a physical property, e.g., electrical conductivity or refractive index (see Abstract). **Gold** *et al.* teach that the nucleic acid ligands in such a method are synthesized by the method disclosed in Pitner et al. as cited herein (col. 15, ll. 44-49). **Gold** *et al.* further disclose that a fluorophore such as fluorescein or Texas Red may be attached to the ligand on the biochip and binding of the target can be determined by measuring a

change in fluorescence intensity, fluorescence polarization, fluorescence anisotropy and fluorescence lifetime (col. 15, lines 42-65).

Applicant has amended claims 1 and 15 to incorporate the covalent coupling limitation recited in claim 5 and incorporated the same limitation recited in claim 17 into claim 15, respectively. Thus the claims recite a step of covalently coupling the reporter molecule/fluorescent dye to the aptamer to form the signaling aptamer. Additionally, the limitations recited in claim 18, drawn to the reporter molecule/fluorescent dye being inserted between two nucleic acid residues or replacing a nucleic acid residue, are incorporated into claim 15 and these limitations added to claim 1. Claims 5 and 17-18 are canceled.

As stated by the Examiner, neither **Pitner** *et al.* nor **Gold** *et al.* teach where the reporter molecule/fluorescent dye is placed within the aptamer, although Pitner et al. teaches that the reporter is covalently linked or tethered to a residue The Examiner also states that both the ligand (target molecule) and the signaling aptamer are in solution (col. 9, ll. 10-13). As recited in amended claims 1 and 15, the reporter molecule/fluorescent dye in the instant aptamers are not tethered to a nucleotide.

Gold *et al.* specifically teach immobilizing or linking their fluorescently labeled nucleic acid ligands (the instant signaling aptamers) to a solid support or biochip (col. 15, lines 42-46). Applicants specifically teach that the signaling aptamer may be in solution or may be immobilized on a solid support (pg. 13, ll. 10-12). The instant application further teaches that having a signaling aptamer in solution is not the same as immobilizing a signaling aptamer because the signaling aptamer in solution can directly signal the presence of a ligand or target in the solution without prior immobilization or washing steps (pg. 28, ll. 8-10).

As such, a signaling aptamer is homogenously in solution, i.e., is free to distribute homogenously. **Gold** *et al.* teach that a nucleic acid ligand is immobilized on a biochip in a manner conducive to binding a target molecule upon the nucleic acid ligand contacting a solution comprising the target molecule (col. 19, ll. 31-55). The immobilized nucleic acid ligand is not in solution as solution is defined in the art. However, such distinctions notwithstanding, what is significant is that neither **Pitner** *et al.* nor **Gold** *et al.* teach a signaling aptamer in which a nucleotide is replaced with a reporter molecule/fluorescent dye or inserted between two nucleotides.

Regarding dependent claims 2, 5-12, 17, 19, 23, and 25, Applicant has canceled claims 2, 5 and 17. The remaining dependent claims are drawn to the types of molecules used for the aptamers, reporter molecules and dyes, specific types of signaling aptamers and a method of quantitation using the methods recited in independent claims 1 and/or 15. As these claims depend from amended independent claims 1 or 15, neither **Pitner** *et al.* nor **Gold** *et al.* can anticipate these claims because neither **Pitner** *et al.* nor **Gold** *et al.* anticipate amended independent claims 1 and 15.

For a valid §102 rejection, the prior art references must contain each element of the claimed invention. Absent the teaching of covalently coupling the reporter/fluorescent dye between two nucleic acid residues or covalently coupling the reporter/fluorescent dye by replacing a nucleic residue to form the signaling aptamer neither **Pitner** *et al.* nor **Gold** et al. anticipate Applicant's claimed invention. Therefore, as this reference is not valid prior art against the instant application under 35 U.S.C. §102 and in view of the preceding amendments and remarks, Applicant respectfully submits that the cited references do not anticipate claims 1-2, 5-12, 15, 17, 19, 23, and 25 under 35 U.S.C. §102. Accordingly, Applicant respectfully requests that the rejection of

claims 1-2, 5-12, 15, 17, 19, 23, and 25 under 35 U.S.C. §102(b) and §102(e) be withdrawn.

The 35 U.S.C. §103(b) rejections

Claims 18, 20-22, and 24 are rejected under 35 U.S.C. §103(a) as being unpatentable over **Pitner** *et al.* as applied to claims 1-2, 5-12, 15, 17, 19, 23, and 25 above, and further in view of **Gold** *et al*, **Conrad** (U.S. 5,728,525) and **Szostak** *et al.* (U.S. 5,631,146). Claim 28 is rejected under 35 U.S.C. §103(a) as being unpatentable over **Pitner** *et al.* as applied to claims 1-2, 5-12, 15, 17, 19, 23, and 25 above and further in view of **Royer.** Applicant respectfully traverses this rejection.

With regard to claim 18, the Examiner states that it would have been obvious and the skilled practitioner would have been motivated at the time the claimed invention was made to label the nucleic acid ligand of **Pitner** *et al.* by replacing a nucleic acid residue with a fluorescent dye as disclosed in **Conrad** (col. 12, ll. 46-53) by replacing the residue during chemical or enzymatic synthesis. **Pitner** *et al.* is as described *supra.* **Conrad** teaches nucleoside analogs which are **inherently** (Applicant's emphasis) fluorescent (Abstract) and are useful as monomers in synthesizing

and labeling nucleotide sequences. These monomers can substitute for naturally occurring nucleosides in the synthesis of oligonucleotide probes which are useful in oligonucleotide amplification, detection, identification, and/or hybridization assays (Abstract; col. 6, lines 45-51).

Although claim 18, which depended from independent claim 15, is canceled, the limitations of the claim are incorporated into both claims 1 and 15 to overcome the novelty rejections discussed *supra*. In considering what is fairly taught in **Conrad**, one of skill in the art would be motivated to prepare and use the inherently fluorescent nucleoside analogs to synthesize a fluorescent oligonucleotide to use as a probe. However, because these analogs are autofluorescent, no change in fluorescence is detected upon the probe binding to a complementary sequence; the appearance of fluorescence in a recovered product simply indicates that a sequence complementary to the fluorescent probe was present or synthesized or hybridized depending on the particular use for the probe.

Therefore, this would not be an effective method of transducing a conformational change to an increase in fluorescence. Furthermore, **Conrad** does not teach a fluorescent probe

comprising a fluorescent dye. In fact, no motivation to do so is found in **Conrad** because the novel fluorescent nucleoside analogs are already inherently fluorescent. Applicant respectfully submits that obviousness can not be established by combining the teachings of the prior art absent some teaching, suggestion or motivation supporting the combination to do so. Thus, absent the suggestion or motivation in **Conrad** to covalently couple a fluorescent dye within an oligonucleotide (aptamer) one of ordinary skill in the art in combining **Conrad** with **Pitner** *et al.* would not have Applicant's invention.

Gold *et al.* is as previously described. **Szostak** *et al.* teach single-stranded DNA molecules which bind adenosine or an adenosine-5'-phosphate and methods for producing and isolating them. **Royer** teaches a method of quantitation of a macromolecule in solution by measuring the changes in fluorescence polarization upon the association of the macromolecule with an oligonucleotide labeled with a fluorophore covalently coupled to the oligonucleotide (Abstract; claim 1). This is essentially the method of **Pitner** *et al.*

Claims 20-22, 24 and 28 depend from amended independent claim 15. Claims 20-22 and 25 limit the aptamers and ligands used in the instant method. Claim 28 limits the method by

adding an additional step of quantitating the ligand through the increase in fluorescence intensity or colorimetric intensity. To reiterate, the incorporation of claim 18 into claims 1 and 15 confers novelty on the instant invention, as recited in these amended claims. Furthermore, claim 18 does not render the instant invention obvious by the combination of **Pitner** *et al.* with **Conrad**. As such, further combinations of **Pitner** *et al.* and **Conrad** with **Gold** *et al.* and **Szostak** *et al.*, claims 20-22 and 24, and with **Royer** *et al.* (claim 28) also do not render the instant invention obvious. Thus, the invention as a whole was not obvious to one of ordinary skill in the art at the time the invention was made. Accordingly, Applicant respectfully requests that the rejection of claims 18, 20-22, 24, and 28 under 35 U.S.C. §103(a) be withdrawn.

This is intended to supplement the response to the Final Office Action mailed November 26, 2002. If any issues remain, the Examiner is respectfully requested to telephone the attorney of record signing the instant document for immediate resolution. Please debit the $375 fee under 37 C.F.R. 1.17(e) to file a Request for Continued Examination or any additional applicable fees due from Deposit Account 07-1185. As the Examiner has set the period

of reply to the Advisory Action to expire August 26, 2003, Applicant believes no additional extension fees are due.